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Nanoparticles as Nano-priming Agent for Antifungal and Antibacterial Activity against Plant Pathogens

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ABSTRACT: Engineered nanoparticles have become important in the areas of nanotechnology which entered into all aspects of human life now-a-days. In field of agriculture also, nanotechnology has been used. Nanoparticles have promising solution over traditional agricultural practices. In seed technology long term storage and field stand are the major challenges which is very difficult to handle. Seeds nanopriming enhances seed germination and improves crop productivity. It also improves the biochemical parameters of the seeds. Nanoparticles also have antifungal and antibacterial activity against many fungal and bacterial plant pathogens. In this article we reviewed the importance of nanopriming for better plant health management. In this review article emphasis has been given on the how the nanoparticle can be better used for as priming agents for better seed storage, field stand without any negative effect on the biochemical parameters. The recent achievement on use of nanoparticle as nanopriming agent with antimicrobial activities has been reviewed.

Keywords: Antibacterial activity, antifungal activity, nanopriming, nanotechnology

INTRODUCTION

Nanotechnology is the combination of different disciplines of art and science. In this science, scientist design, characterize, produce of structures, devices and systems by controlling shape and size on the nanoscale as per the need of its application (Dutta and Kaman, 2017). Generally useful materials are created at nanoscale with novel properties (physical, chemical, biological) at that scale. In recent years, the science is considered as emerging as innovative technology where the scientist with expertise in physics, chemistry, biology, material science and medicine are work together with a definite goal.

Now-a days, engineered nanoparticles become most important index in the area of nanotechnology which has entered into all aspects of human life and their various applications that can be quickly expanded as compared to bulk materials due to their new characteristics. There are many reports in which it is stated that biological processes have been improved by using engineered nanoparticles (Husen and Siddiqui, 2014).

Nanotechnology has been also used in the field of agriculture. It is used in controlling the plant pathogens for which the productivity and production is reduced every year worldwide due to plant diseases. Widely pesticides cause the environmental hazards due to which the researchers developed a new technology, named as "nanotechnology" to manage pathogens (Jo *et al.*, 2009). Nanotechnology has a potential to improve the farm productivity and it can minimise the cost of resources and environment that are related to agricultural sectors (Dutta, 2012). Nanomaterials can be used in different areas of agriculture. It can be useful for crop improvement, management of water and weed, seed and food technology etc.

A. Nanopriming

Agriculture and food sector are related to human life and therefore now-a-days, the use of nanotechnology has become important in agriculture sector and promising solution over traditional agricultural practices. It also improves crop productivity and quality. Seed nanopriming increases crop productivity in field condition (Shukla, 2019). It also enhances seed

germination, increase all physiological parameters both in storage and in field condition.

"Nanopriming" is a new method of seed priming in field of agriculture where nanoparticles like Zinc Oxide, silver nanoparticles, gold nanoparticles etc. are used. Seed nanopriming is done to improve germination percentage, seedling growth and increasing seedling vigour index in the field of agriculture.

Nanopriming was significant on germination of sunflower. The sunflower seeds were treated for 10 and 20 minute with nanoparticles. Then the seeds were kept in a petriplates for germination. After 8 days, germination percentage was recorded and found that seeds treated for 20 minutes achieved highest germination percentage, seedling dry weight and seedling vigour index as compared to 10 minutes treated seeds and control. Hence, it was concluded that germination percentage of seeds increased with increasing nanopriming time (Maroufi *et al.,* 2011).

Nano iron- chelated fertilizer increased the seed number per pod, pod number per plant, 100 seed weight and grain yield of chickpea as compared to control by 17, 48, 13 and 65% respectively. Seed priming with Zn, Fe and Ca increases the 100 seed weight and grain of chickpea than non- priming seeds (Valadkhan *et al.,* 2015).

Nanopriming with gold nanoparticles at a concentration of 5 ppm helps to activate maize seed germination and promotes emergence percentage as compared to unprimed control and hydroprimed groups (56%) with 3 times more seedling vigour than the control (Mahakham *et al.,* 2016). Husen and Siddiqi (2014) reported that nanopriming with engineered nanoparticles helps in increasing the biological process of plants.

Krishnaraj *et al.,* (2012) reported that ZnO NP treated seeds showed increased germination per cent which may be due to stimulation of antioxidants system. Increased plant growth parameter like root and shoot length, fresh and dry weight of roots and shoots in ZnO NP @100 ppm treated plants were also reported by Kaushik, (2019).

ZnO NP has positive impact on seed germination, seedling vigour and growth of tomato at 400 ppm (Khanm *et al*., 2018). Zafar *et al*., (2016) reported that treatment with ZnO NP increase the biochemical parameters in *Brassica nigra* seedlings. Mohsenzadeh and Moosavian (2017) showed that ZnO NP can increase the amount of soluble sugar in treated plant as compared to that of control.

Pawar *et al*., (2019) reported that iron oxide nanoparticles (Fe_2O_3 NPs) enhanced the seedling growth of chickpea of variety *Digvijay*. Seeds were primed with iron oxide nanoparticles with different concentration ranging from 4 to 16 microgram/ml and starch was taken as a coating agents. Primed seeds were air dried and later used to study the seedling growth by using paper towel method for *in vitro* as well as *in vivo.* The growth parameters like root length , shoot length etc were considered as crucial indicators. These growth parameters indicates that seeds enhanced in lower concentration upto 12 microgram/ml and inhibits further growth at higher concentration. Hence, Fe_2O_3 NPs at optimised dose level can be used as co-fertilizers to improve the chickpea growth in lower concentration.

Prazak *et al*., (2020) reported that under normal and chill temperature silver nanoparticles (AgNPs) have positive effect on germination, field emergence and physiological parameters of two variety of bean namely Bali and Delfina. AgNPs solution was applied as a short-term pre-sowing treatment at a concentration of 0.25, 1.25 and 2.5 mg dm^{-3} along with the microbial agent Nitragina which contains *Rhizobium leguminosarum* pv. *phaseoli*. Lower concentration of AgNPs have beneficial effect on seed germination and physiological parameters in both laboratory and field condition. Black gram seeds priming with Zinc oxide (ZnO) and Copper (Cu) nanoparticles improves the seed quality (Raja *et al*., 2019).

Seed priming with 60 ppm titanium dioxide can effectively enhance the seed germination and plant growth parameters maize seedling under abiotic stress condition like salinity stress. The growth parameters and relative water content, biochemical parameters like total phenolic and proline contents, catalase (CAT), phenylalanine ammonia lyase (PAL) etc were found significantly increased and on the other hand sodium ion concentration, mean emergence time, membrane electrolyte leakage and melondialdehyde were decreased when titanium dioxide was used as priming agents as compared to control under salinity stress (Shah *et al.,* 2020).

Using biosynthesized Zinc oxide (ZnO) nanoparticles as a nanopriming agent can significantly enhance the germination per cent of seed and growth parameters of maize than ionic control (Zinc acetate) and hydropriming. ZnO nanoparticles was absorbed on the endosperm region of the seed which was revealed by HR-SEM analysis. ZnO NP can reduce the Zn deficiency and improves the agronomic characters of the maize seeds (Itroutwar *et al*., 2020).

B. Antifungal and antibacterial effect of nanoparticles

In three types of media, 3 types of nanosilver liquid (WA-CV-WA13B, WA-AT-WB13R and WA-PR-WB13R) were used against *Sclerotium cepivorum* which causes white rot of green onion in different concentration as reported by Jung *et al*., (2010). The results showed that all nanosilver liquid inhibit the pathogen more than 90% at a concentration of 7 ppm. In *in vivo,* it showed that nanosilver liquid developed the plant growth by increasing the biomass and dry weights and it decreases the population of pathogen in soil.

Spherical silver nanoparticles of size 10, 20, 40 nm have antifungal effect against *Tricophyton rubrum* and some fungus like *Aspergillus*, *Candida* and *Albicance* as reported by Aghamoosa and Sabokbar (2014).

Alananbeh *et al.,* (2017) studied the effect of Ag nanoparticles on fungi isolated from raw and waste water. In this study, they have collected the water from 2 sources: home and hospital, additionally bottled water and autoclave distilled water was used as a control. Fungi were isolated from the sample of water and purified on Potato Dextrose Agar (PDA). Eight genera and nine species of fungal were identified: *Aspergillus*

flavus, A. niger, A. terreus, Fusarium oxysporum, F. solani, Geotrichum candidum, Mucor hiemalis, Penicillium chrysogenum, Rhizopus oryzae, Trichoderma harizianum and *Trichophyton* sp. *Aspergillus* sp were found to be highest and selected for further experiment. On *Aspergillus niger* and *Aspergillus terrus*, silver nanoparticles of rod shaped and cube shaped were tested at a concentration of 1, 10 100µg/ ml. Results showed that increase in concentration of silver nanoparticles, reduced the gradual growth of both the *Aspergillus* sp. It was found that *Aspergillus terrus* have higher reduction as compared to *Aspergillus niger.*

The antimicrobial activity of two bio- synthesized NPs i.e., gold and silver were studies against four human pathogens viz. *Bacillus cereus*, *Escherichia coli*, *Staphyllococcus aureus* and *Aspergillus niger*. It was found that biogenic Ag nanoparticles showed a strong antimicrobial activity against *B. cereus* followed by *E. coli*, *S. aureus* and *A. niger* as 26.13, 26.02, 25.43 and 22.69 respectively (Bhuyan *et al.,* 2017). They also reported that silver NPs may be due the their small size (5–25 nm) could easily penetrated into the cell membrane and there by cause disturbance of the metabolism, cause irretrievable damage and ultimately leads to cell death. But, the biogenically synthesized gold nanoparticles didn't show any antimicrobial activity

In another study Das and Dutta (2021) evaluated *Trichoderma asperellum* mediated Ag and Au nanoparticles as antifungal activity against the fungal pathogen, *Rhizoctonia solani* causing sheath blight of rice. The nanoparticles were tested at 6 different concentration (viz., 1, 5, 10, 50, 100 and 200 ppm) and compared with the chemical. Result showed that Ag NP at 200 ppm showed maximum radial growth inhibition per cent with 73.39%. Au nanoparticle at 200 ppm caused a 60.83% inhibition of the pathogen. Electron microscopy study showed that Ag NP accumulate inside the fungal cells thereby cause distortion of fungal cells leading to death of the pathogen and found as these as the main mode of action. While studying the effect of Ag NP at 200 and 100 ppm on sclerotia it was found that Ag nanoparticle caused complete inhibition of germination of sclerotia of *R. solani*. In pot experiment, Ag NP at 200 ppm showed increased the plant growth parameters with 20.00% reduction of disease incidence as compared to control *R. solani* where they recorded 88.00% disease incidence with increased the concentration of vital secondary metabolites like phenols, flavonoids, terpenoids and total soluble sugars in the Ag nanoparticle treated pot. While studying the effect of fungus mediated silver nanoparticles against inoculum, biomass and protein content of *Fusarium oxysporum* Dutta *et al*., (2020) found significant reduction of inoculum concentration, biomass and protein content of the pathogen and thereby causing death of the fungus.

Silver nanoparticles have antifungal activity against *Trichosporon asahii.* They studied the growth of *Trichosporon asahii* against different concentration of silver nanoparticles. Result revealed that inhibitory growth of silver nanoparticles against *Trichosporon asahii* was minimum at a concentration of 0.15µg/ml and based on electron microscopy,silver nanoparticles permits the funal cell and damage the cell wall and cellular components of *Trichosporon asahii* (Xia *et al.,* 2016).

Ouda (2014) studied the inhibitory properties of silver nanoparticles against *Alternaria alternata* and *Botrytis cinerea* at various concentration. The result revealed that silver nanoparticles have highest inhibitory growth at a concentration of 15 mg L^{-1}. Microscopic observation shows that silver nanoparticles damage the effect of fungal hyphae and conidia. AgNPs also reduced the effect of sugar, protein of both the pathogen.

Dimkpa *et al.,* (2013) studied the potentiality of ZnO nanoparticles (NPs) and bacterial biocontrol agent against growth of *F. graminearum*. In this study significant fungal growth inhibition was observed which was caused by ZnO NPs and inhibition per cent was better than micro-sized particles of ZnO. From this study it was understand that though both types of particles released similar levels of soluble Zn but toxicity capacity is dependant on the size of the particles.

Yehia *et al.,* (2013) studied the antifungal activity of ZnO NPs against *F. oxysporum* and *P. expansum* and found that the antifungal activity is concentration dependent. They found highest mycelial growth inhibition at 12 mg L^{-1}, with 77% and 100% mycelial growth inhibition for *F. oxysporum* and *P. expansum*, respectively.

George *et al.,* (2014) studied the anti-fungal activity of ZnO and TiO_2NPs against the fungal pathogens like *Aspergillus niger, Trichophyton, Fonsecaea, Aspergillus flavus, Rhizopus oryzae, Fusarium, Ramichloridium schulzeri and Cladosporium*, on agar and in broth media and comparison was made with two common antifungal compounds Amphotericin-B and Miconazole. They reported that both the nanoparticles were highly effective than the bulk-particles and as efficient as Amphotericin-B. But, at equal dose Miconazole was found to have a better anti-fungal activity. While comparison was made it was found that ZnO NPs better anti-fungal than TiO_2.

Boruah and Dutta (2021) synthesized chitosan nanoparticles from a fungal pathogen *viz*., *Fusarium oxysporum*, and three fungal bioconbtrol agents, viz., *Metarhizium anisopliae, Beauveria bassiana* and *Trichoderma viride* and from a commercial source of chitosan. Studies on characterization of the synthesized NPs showed absorption peaks at the range of 310.02 to 342.00 nm in UV–VIS spectroscopy study. Presence of OH, N–H, C–H, C=O, C–O, C–N and P=O were confirmed by FTIR study which are considered as the functional groups of chitosan nanoparticle. The NPs were found to have positive charge and were found stable in nature with nearly spherical in shape (as comfirmed electron microscopy study). The average size of the chitosan nanoparticles were found as 273.20, 172.50, 78.36, 89.03 and 300.10 nm for *F. oxysporum, M. anisopliae, B. bassiana, T. viride* and commercial products respectively. The synthesized chitosan nanoparticles were found to be highly compatible with

T. Asperellum at the three different tested dosage. While studying the *in vitro* efficacy three soil borne plant pathogens viz., against *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii*, the combination of *T. asperellum* and chitosan nanoparticle was found as the best treatment combination for in inhibiting the suppression of radial growth of the pathogens as compared to *Trichoderma* alone and at 0.1% carbendazim.

Goswami *et al.* (2020) studied the antifungal efficacy of biosynthesized silver nanoparticles (BSNPs) against the pathogen causing crown rot and anthracnose of banana caused by *Colletotrichum musae* at different concentrations (0.0001%, 0.001%, 0.01%, 0.1% and 0.2%) and compared with chemical fungicide (Carbendazim @ 0.1%). BSNPs at a concentration of 0.2 per cent effectively inhibited radial growth of the pathogen. The effect of BSNPs against anthracnose and crown rot diseases of banana was also studied by undertaking five treatment combinations. Among all the treatments, pre harvest spray of 0.2 per cent BSNP one week prior to harvest alongwith hands dip treatment of harvested banana fruits in BSNPs for 10 min was best for anthracnose disease with a per cent disease index (PDI) of 51.72 against 97.17 in control after 15 days of harvest. In case of crown rot, highest PDI of 94.38 was recorded in control against 56.90 in the same treatment with BSNPs for 10 min was best for anthracnose disease with a per cent disease index (PDI) of 51.72 as against 97.17 in control after 15 days of harvest. In case of crown rot, highest PDI of 94.38 was recorded in control against 56.90 in the same treatment with 0.2 per cent of BSNPs . The highest shelf life of 15 days after harvest was recorded when banana fruits were treated with 0.2 per cent of BSNPs both as pre-harvest spray and post-harvest application against 8 days in control. Spraying of recommended dose of Carbendazim (0.1%) can increase the shelf life of banana fruits upto 14 days only.

Pegu *et al*., (2019) studied the effect of chemically synthesized Zinc Oxide nanoparticle as nano priming agents to improve the field performance of direct seeded rice for early seedling vigour and improvement in yield. They reported highest spikelet fertility (82.17%), seedling vigour index (3479.16), seedling height (42.15 cm), Chl a (9.73), Chl b (3.89), pollen fertility (90.13%) etc. with ZnO an nanopriming agent. Kaushik and Dutta, 2017; Dutta *et al*., 2021 during their study on efficacy of ZnO particle found effective result in suppressing the mycelial growth of *Sclerotinia scleritiorum* and *Rhizoctonia solani* at 50, 100 and 200 ppm concentration.

Karimiyan *et al.*, (2015) studied the antifungal effects of ZnO NPs by investigating *in vitro* against the pathogen *Candida albicans* and this NPs was compared with amphotericin B. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of these nano-particles were evaluated. Acetic acid solution was prepared from the suspension of nanoparticles. Results showed that MIC and MFC of nano-ZnO was recorded 200 μgmL^{-1} and 400 μgmL^{-1}, respectively. The MIC and MFC of amphotericin B was 0.5 μgmL^{-1} and 2 μgmL^{-1}, respectively. The overall experiment summerized that, ZnO have anti *C. albicans* activity and it can be used for treating of the infections disease caused by *C. albicans* but further *in vivo* investigation in needed.

Jo *et al.,* (2009) reported that Ag ions and NPs have antifungal activity against *Magnaporthe grisea* and *Bipolaris sorokiniana*. In *in vitro* assays, the nanoparticles showed significant effect on colony formation against both pathogens.

Qi *et al*., (2004) reported that chitosan NPs and Cu-loaded NPs can inhibit the growth of various bacteria namely, *E. coli, S. aureus, S. tymphimurium* and *S. choleraesius.* Ag NPs, synthesized from gallic acid aqueous solution with different sizes (7,29 and 89 nm mean value) have antibacterial activity. The antibacterial activity varies with the size dimensions of the nanoparticles (Martínez-Castañon *et al.,* 2008).

Silver nanoparticles (AgNPs) have antibacterial activity on *E. coli.* AgNPs can harm the bacterial structure and slow down the activity of some membranous enzyme (Li *et al.,* 2010). Kaviya *et al.,* (2011) reported that biosynthesis of AgNPs from *Citrus sinensis* peel extract have antibacterial activity against *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Synthesis of AgNPs was done at room temperature (25°C) and formation of silver nanoparticles was confirmed by using TEM, FESEM, EDAX, RTIR, XRD and UV-vis analysis.

Emami- Karvani *et al*., (2011) reported the antimicrobial activity of ZnO NPs against gram-positive and gram-negative bacteria. *E. coli* and *S. aureus* were taken to test against ZnO by disc and well diffusion agar method and result showed that gram-negative bacteria was seemed to be more resistant to ZnO than gram-positive bacteria.

Chidambaram *et al*., (2012), synthesized Au NPs using aqueous extract of seed of *Abelmoschus esculentus* and characterized for crystallinity, shape, size, potentiality, charge functional groups, etc. Antifungal activity of Au NPs were tested against *Puccinia graministritci*, *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* using standard well diffusion method. Au NPs causes highest zone of inhibition against *P. graminis* and *C. albicans* with 17 mm and 18 mm respectively.

Shabani *et al.,* (2016), synthesized Au NPs, spherical in shape, from *Fusarium oxysporum* with an average size of 20 nm determined by scanning and transmission electron microscopy and dynamic light scattering and found antifungal properties against human pathogenic yeasts and molds by inhibition zones ranged from 10 to 18 mm.

Savi *et al*., (2012), studied the antifungal activitiy of Au NPs against three pathogenic fungi, *Fusarium verticillioides, Penicillium citrinum* and *Aspergillus flavus* at a concentration of 0, 0.05, 0.1 and 0.2 mg L^{-1}, which was evaluated at 2, 4, 6 and 8 days after incubation and found that with the increased in concentration, the diameter of the fungal colony was decreased.

C. Mode of action of gold nanoparticle

Wani and Ahmad (2013), reported that Au NPs can cause intracellular acidification due to inhibition of H^+-

ATPase activity ultimately leading to death. On the other hand Tiwari and Lee (2013) reported that Au NPs attaches itself to the membrane of bacteria and the integrity of cell is disturbed. It also alters the membrane potential by deterring the activity of ATPase enzyme resulting in lower concentration of ATP inside the cell and also disrupts the translation process by preventing the binding of tRNA to the ribosomal subunits (Cui *et al*., 2012)

Au NPs cause outflow of the contents of the cell and subsequently increasing the permeability of the cell wall due to creation of holes in it and ultimately death of bacteria and also prevents transcription to occur by binding with the DNA (Rai *et al* ., 2010)

Yu *et al., (*2016), reported that Au NPs do not allow the formation of bacterial biofilm which is essential for its pathogenicity and directed the stimulation of immune response-related genes. Another study conducted by Vijaykumar and Ganesan in 2012 reported that Au NPs bind with gp 120, a glycoprotein and prevent the attachment to CD4 receptor of the virus, thereby preventing its entry. When Au NPs interact with virus cell, they create pressure that destroy the shape of the virus cells, deforms it and opens it rendering it harmless. Against viral pathogens Au NPs block the viral infection by stopping its entry, its attachment to the host cells and cell to cell movement (Baram -Pinto *et al* ., 2010).

D. Role of nanoparticle on biochemical defence mechanisms of plants

Literature related to role of nanoparticle on biochemical defence mechanisms of plants on Au NPs is rare.Yasur and Rani (2013) studied the impact application of AgNPs and $AgNO_3$ on seed germination, growth parameters of castor bean, *Ricinus communis*. No significant effects on seedling growth even at 4,000 mg L^{-1} of AgNPs, while in bulk form of Ag as $AgNO_3$ inhibited the seed germination of castor. Through atomic absorption spectroscopy the uptake of Ag in seedlings of the castor seeds was confirmed. An enhanced enzymatic activity of ROS enzymes and phenolic content in castor seedlings was observed AgNPS and $AgNO_3$ treated plants. Besides, enhanced content of individual phenols i.e., aprahydroxy benzoic acid was found through HPLC analysis.

Raigond *et al*., (2017) used two foliar sprays of ZnNPs at three different concentration viz., 100, 300 and 500 ppm on potato plants, first at 25 days and the second at 45 days of planting and reported that the high activity of enzymes in Zn NPs treated plants compared to control. They reported 142,109 and 212% increase in catalase activity in Zn NPs treated plants at 100, 300 and 500 ppm, respectively. Significant increase in peroxidase activity was reported at 500 ppm. Besides, increased total phenolics and anthocyanin content at 300 and 500 ppm concentrations of Zn NPs were recorded. While analysing the starch content it was found 15, 29 and 259% increase dueat 100, 300 and 500 ppm Zn NPs., respectively. The enhanced in both enzymatic and non-enzymatic antioxidants revealed that the Zn NPs lead to oxidative stress and caused toxicity in potato plants.

Chandran *et al.*, (2015) studied the effect of chitosan nanoparticle on innate immune responses in *Camellia sinensis* plants. They found that upon treating the leaves with nanoparticle, there was an increase in the activity of defence enzyme i.e., increase in accumulation of PO (Perioxidase), β-1, 3-glucanase, PPO (Poly Phenol Oxidase) and PAL (Phenylalanine ammonia lyase) as compared to treatment with chitosan. Antioxidant enzyme like superoxide dismutase (SOD) and catalase (CAT) were found to increase by 41% to 49% in case of chitosan nanoparticle treatment whereas 33% to 40% increase was recorded when plants were treated with chitosan alone. The recorded 3.5% higher induction of phenol content in the leaves treated with chitosan NPs than chitosan. Similarly flavonoid content was also found to be increased in the chitosan nanoparticle treated leaves as compared to chitosan. Transcript analysis showed higher expression of PPO, β-1, 3-glucanase, PAL and TLP genes in chitosan NPs treated leaves than that treated with chitosan. Also they found higher levels of PO generation in both the chitosan and chitosan NPs treated leaves compared to the untreated leaves.

CONCLUSION

Nanopriming reduced the difference between the time of sowing and seedling emergence and improve the germination rate and total germination percentage. Priming with nanoparticles can also increase the shoot and root length, improve fresh and dry weight. Nanoparticles are also have a great potential against fungal and bacterial pathogen. It can inhibit or suppress the growth of fungi and bacteria in both *in vitro* and *in vivo* condition. In the future the cited work can utilized by the research scholar and scientific community for the better management of seed related problem with better plant stand in the field condition.

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