BCIL Biotech Consortium India Limited

# Development of lignin-based nano-biofertilizers and nano-biopesticides and their applications

**TECHNOLOGY AVAILABLE FOR TRANSFER**

## UNMET NEED AND OPPORTUNITY

The use of conventional pesticides and fertilizers leads to health hazards for farmers due to toxicity. Additionally, the application of large quantities in fields leads to soil and environmental pollution. The use of eco-friendly agrochemicals is therefore of utmost importance.

***The present invention uses nanotechnology to develop nano-formulations as a viable delivery carrier. These formulations are made of lignin which is biocompatible and biodegradable. Moreover, the nanoparticles reduce the quantity of pesticides and fertilizers to be used due to high surface area-to-volume ratio.***

## TECHNOLOGY

In the present invention lignin was used as a catalyzing, capping and stabilizing source to synthesize zinc oxide nano composites [ZnONCs] i.e. (nano-biofertilizer ZnOKL), lignin derived nano urea (nano-biofertilizer) and lignin derived nano- biopesticide (Azadirachtin) through a low cost, green and in one pot/single step. The present invention uses nanotechnology and environmentally friendly methods to develop nano-biofertilizer and nano-biopesticides in a cost-effective manner.

More than 99% of pesticides fail to reach their targets and leave harmful impacts on soil, water, and air health while increasing pathogenic resistance and reducing biodiversity. However, in the present invention the utilization of lignin derived nanoparticles reduces the quantity of pesticides and fertilizers to be used as the nanoparticles, increases the surface-to-volume ratio and act precisely on target. Since lignin has a wide range of properties including antioxidant, antimicrobial and UV protectant, the lignin derived ZnONC (ZnOKL) showed promising antioxidant potential compared to the commercial ZnO nanoparticles (ZnONPs) and pure ZnO. Moreover, this nano composite showed good anti-microbial activity against Gram negative (*E. Coli*) and Gram positive bacteria (*B. Megaterium*) compared to commercial ZnONPs and pure ZnO.

## UNIQUE SELLING PROPOSITIONS

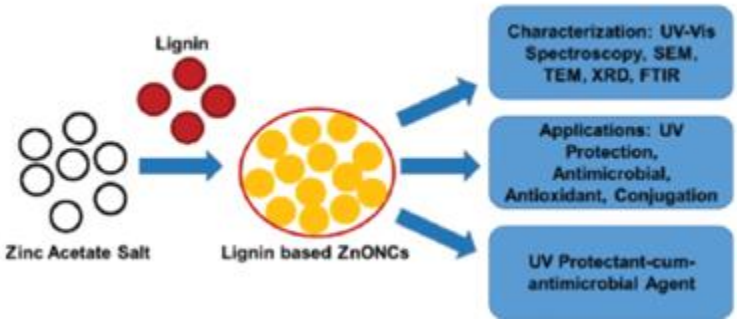
- **Quality** – Biocompatible and biodegradable.
- **Enhanced efficacy** – higher loading efficiency.
- **Stability**- Long term stability.
- **Cost effective** –lignin serves as a cost-effective resource and is expected to be of low cost. Cost will vary depending upon the lignin source and the type of bioactive agent used.
- **Shelf life** – Lignin based nano-composites are stable for 6 months at room temperature and 1 year at 4ºC.
- **Ease of use** – easy to prepare, biocompatible, can be sprayed over the soil
- **Toxicity**- Lignin-based nanoparticles were found to be non-toxic in in-vitro studies

## PROPERTIES OF NANO-BIOFERTILIZER

**Lignin loaded Nano-composite:**

In comparison to commercial ZnO-Alkali Lignin (ZnOAL), which had a 25.2% lignin loading, biomass-derived ZnO-Kraft Lignin (ZnOKL) had 67.5% lignin loading showing a better zeta potential (-20 eV) which makes ZnOKL more stable nanocomposites compared to the ZnOAL

nanocomposite (-38 eV). This is due to the higher number of –OH functional groups present in phenylglycerol contributing to lower zeta potential of the ZnOAL nanocomposites. Furthermore, a higher UV absorption maximum (350 nm) leads to better antioxidant and antimicrobial properties in case of the ZnOKL nanocomposite.




**Antioxidant activity:**

Lignin based ZnONCs possess good antioxidant potential compared to native lignin or commercial ZnONPs due to the additive effect of lignin and ZnO. ZnOKL nanocomposite has a scavenging effect of 75.67%, whereas ZnONPs from a commercial source have a scavenging effect of 4.62%. This is due to the size of ZnOKL nanocomposite (2–5 nm), whereas the commercial ZnONPs have a size of 20–30 nm. The smaller size of ZnOKL contributes to higher lignin loading leading to a larger surface area as compared to commercial ZnONPs.

**Antimicrobial activity:**

The antimicrobial activity of the developed nanocomposites was tested against a Gram-negative bacterium, *E. coli*, and a Gram-positive bacterium, *B. megaterium*. Varying concentrations (50–500 mg $mL^{-1}$) of lignin and nanocomposites were used to treat the microbial cells and growth curves were plotted against time.

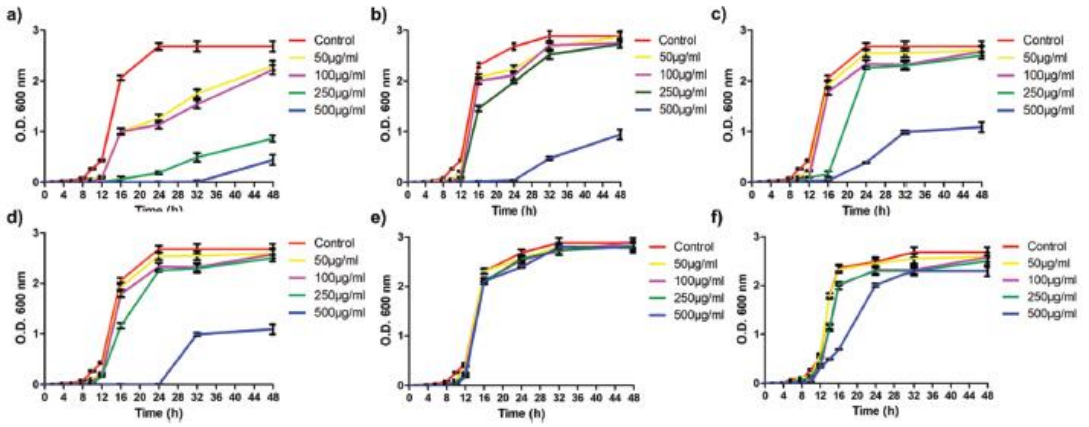



**Fig 1:** *E. coli growth curve in the presence of different concentrations of (a) the ZnOKL nanocomposite, (c) commercial ZnO nanoparticles, (d) kraft lignin, (e) alkali lignin and (f) pure ZnO at different time intervals*

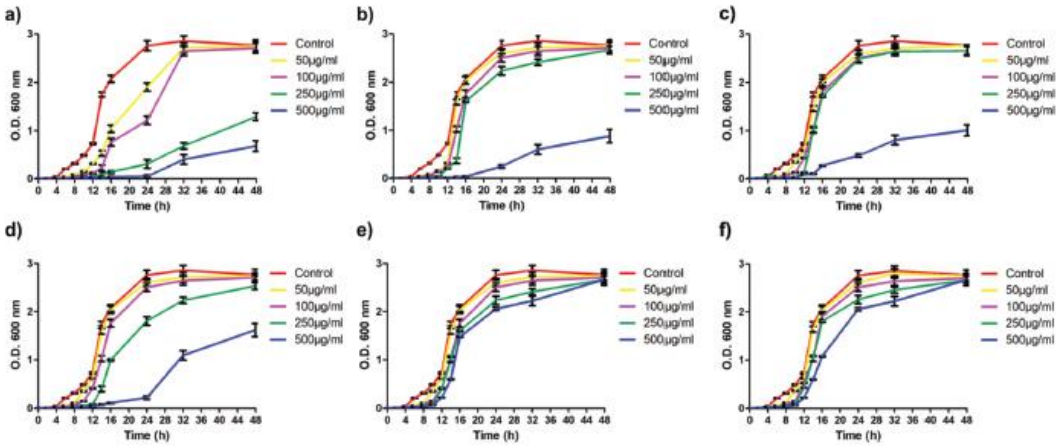



**Fig 2***: B. megaterium growth curve in the presence of different concentrations of (a) the ZnOKL nanocomposite, (c) commercial ZnO nanoparticles, (d) kraft lignin, (e) alkali lignin and (f) pure ZnO at different time intervals*

**Conclusion:** At highest dose of 500 mg $mL^{-1}$ (Fig 1), ZnOKL delayed the growth of *E. coli* for around 32h, as compared to commercial ZnO and pure ZnO nanoparticles which delayed growth of *E. coli* for around 16 h and 8 h, respectively.

Similarly, at highest dose of 500 mg $mL^{-1}$ (Fig 2), ZnOKL delayed the growth of *B. megaterium* for almost 24h, as compared to commercial ZnO and pure ZnO nanoparticles which delayed growth of *B. megaterium* for around 14 h and 8 h respectively.

## Lignin loaded Nano-Urea:

- Nano Urea is a sustainable option for farmers towards smart agriculture.
- It fulfills the plant nutrient requirement as a fertilizer due to its desirable particle size and more surface area making it bioavailable to plants.
- It increases its availability to crop by >80% resulting in higher Nutrient Use Efficiency.
- Lignin loaded Nano urea increases surface-to-volume ratio (Fig. 3) and higher zeta potential (Fig. 4) leading to reduced usage quantity.

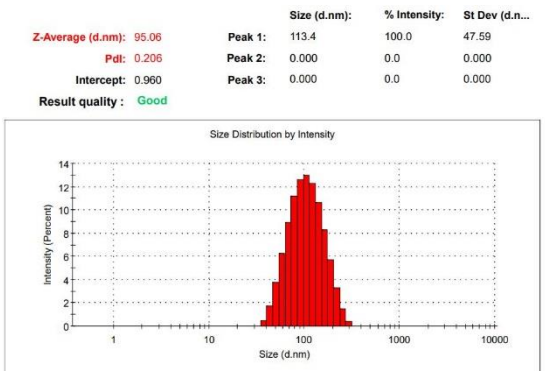



**Fig 3:** Size and PDI of the Nano-lignin Urea (Size ~ 95 nm)

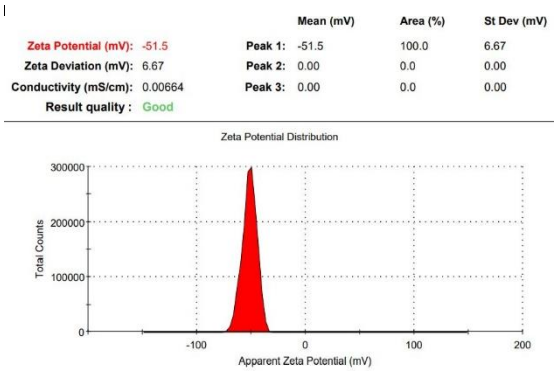



**Fig 4:** Zeta potential of the Nano-lignin Urea (Zeta potential -52 mV)

## PROPERTIES OF NANO-BIOPESTICIDE

**Azadirachtin:**

- Azadirachtin, a secondary metabolite present in neem seeds, is a highly oxidized tetranortriterpenoid.
- The Size and PDI of the Azadirachtin Loaded Nano-lignin is 95 nm (Fig. 5).
- Surface charge of the Azadirachtin Loaded Nano-lignin, Zeta potential -48 mV (Fig. 6)

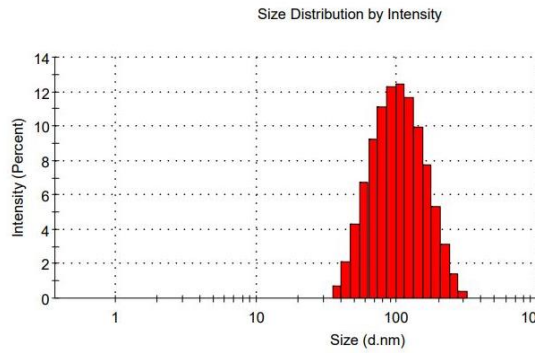



**Fig 5:** Size and PDI of the Azadirachtin Loaded Nano-lignin (Size ~ 95 nm)

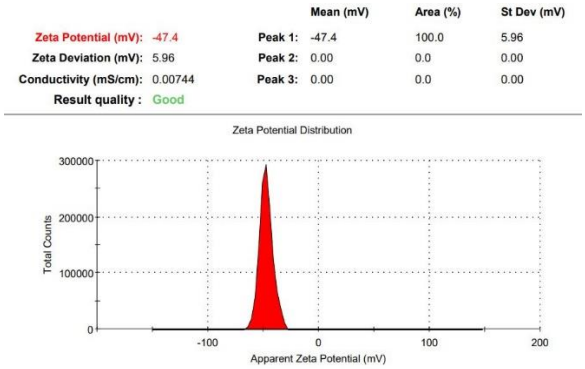



**Fig 6:** Surface charge of the Azadirachtin Loaded Nano-lignin Zeta Potential -48 mV.

**Cytotoxicity:** Being a plant origin, the lignin is easily degradable causing minimal cytotoxic effects. Lignin based polypyrrole nanoformulations exhibit better virus destruction capacity when compared to the standard therapy with minimal cytotoxicity.

## APPLICATION

The agricultural sector, preferably farmers, is the user of this technology. They can spray the nano-biofertilizers and nano-biopesticide for utilization. Due to its biocompatible nature, health hazards for the farmers would be minimal. Less quantity to be used, therefore, cost effective.

## INTELLECTUAL PROPERTY

Patent Application is filed in India.

## LICENSING OPPORTUNITY

BCIL is looking for suitable industrial partner for commercialization of this lignin-based bio-nano technology for agricultural application.

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