CHAPTER 4

SUMMARY AND CONCLUSIONS

In this chapter, a brief summary and conclusions of the results observed from the five sections in Chapter 3 are highlighted.

SUMMARY

The present study focuses on the synthesis and characterisation of silica nano particles from rice husk. The elusive role of silica nanoparticles on improving the soil fertility, silica accumulation, maize growth and physiological characteristics are studied in detail. Additionally, the prophylactic effect of silica nanoparticles is studied based on the degree of disease resistance against fungal pathogens and enhancement of biocontrol action in maize.

4.1 SYNTHESIS, CHARACTERISATION OF SILICA NANOPARTICLES AND ITS TOXICITY ANALYSIS

- Nano silica is synthesised from rice husks and applied as a silica source to soil to study the enhancement of soil nutrients. The prepared silica nanoparticles are comprehensively characterised using different techniques such as XRD, FTIR, SEM-EDAX, PSD, TEM and surface area analysis.
XRD pattern of the prepared silica nanoparticles confirms amorphous nature from the observed broad peak at 22° (2θ). FTIR spectra show the characteristic peaks at 1096 cm\(^{-1}\) and 451 cm\(^{-1}\) corresponding to Si–O–Si and Si–O functional groups respectively. SEM–EDAX results reveal the spherical morphology of the silica nanoparticles with the purity of 98.2%. PSD and TEM analysis confirm the particle size of silica in the range of 20–40 nm with spherical morphology. The obtained surface area of nano silica powder is found high (361 m\(^2\) g\(^{-1}\)).

Before the application of nano silica to food crops (Maize), it is essential to screen the cytotoxicity at different dosage levels. *In vitro* toxicity of nano silica against MG-63 cell line is found to be biologically inert and non-toxic till 125 µg mL\(^{-1}\).

The effect of silica sources on the toxicity of soil is assessed in terms of the enumeration of soil microbial population. Nano silica treatment induces higher population of PSB (3.8 \(\times\) 10\(^4\) CFU g\(^{-1}\)) than that of SSB (2.4 \(\times\) 10\(^4\) CFU g\(^{-1}\)), which is same as that of control (2.0 \(\times\) 10\(^4\) CFU g\(^{-1}\)). The population of nitrogen fixers is considerably high in all the treatments except sodium silicate treatment (2.2 \(\times\) 10\(^4\) CFU g\(^{-1}\)).

While comparing the silica-treated soils without seeds, rhizosphere soils show an increase in microbial population in all the treatments. Nano silica treated soils show the highest population of PSB (4.4 \(\times\) 10\(^4\) CFU g\(^{-1}\)) and nitrogen fixers (4.8 \(\times\) 10\(^4\) CFU g\(^{-1}\)) except SSB, which are
significantly influenced by sodium silicate treatments (2.0 × 10^4 CFU g^-1).

- An increase in C and N ratio in nano silica treated soil (1924:227) and rhizosphere soil (1508:178 μg g^-1) is observed.

- It is seen that nano silica improves the population of soil beneficial microbes, and stabilises soil nutrients over other conventional silica sources both qualitatively and quantitatively.

4.2 ANALYSIS OF SILICA UPTAKE UNDER HYDROPONIC CULTIVATION OF MAIZE SEEDS

- The efficiency of nano silica absorption and its nutritional changes in maize seeds during hydroponic incubation are ascertained. Hydroponic incubation serves as a better route to study the efficiency of silica utilisation by seeds.

- Germination of maize seeds hydroponically treated with nano silica is high (95.5%) while it is reduced (75.77%) under Na_2SiO_3 treatment. No significant difference is observed in micro SiO_2 and H_4SiO_4 treatments. In contrast, there is a considerable increase in the root length of plants treated with nano and micro SiO_2. Hence, the root elongation of plants is expected to have a source dependent response.

- XRF analysis of maize roots shows a remarkable increase in silica content in nano silica treatment (43.5%) than control (33.1%). Thus, the solubility and uptake of nanoparticles greatly affect the cell culture response and radicals.
Nano silica treated seeds reveal good seed viability and hence, it might form complexes and organic compounds in the cell wall of epidermal cells which resist fungi. In contrast, other silica sources make the seeds frailer and affect the seed coat.

The total dry weight of seed ash is higher in nano silica treatment (6.25 ± 0.22 %) than the treatment with micro SiO$_2$ and Na$_2$SiO$_3$. In Na$_2$SiO$_3$ added solution, pH and conductivity are not favorable due to the alkaline pH (9.11).

Thus, the direct uptake of nano silica by seeds is improved in hydroponic incubation that renders potential barrier for maize.

### 4.3 GROWTH AND PHYSIOLOGICAL RESPONSE OF MAIZE UNDER FIELD CULTIVATION STUDIES

Nano silica is added in sandy loam field soil at four different regimes (5, 10, 15 and 20 kg ha$^{-1}$) where maize seeds are sown to explore the morphological and physiological discriminations.

The growth parameters are enhanced in maize with an increase in the concentration of nano silica for the first 20 days. After 20 days, the growth characteristics of maize remain the same in all treated plots including micro silica treatments in soil.

Leaf samples of 20 days old maize reveal a gradual increase in chlorophyll $a$ and $b$ with the concentration gradient of nano silica in contrast to micro counterpart (0.012 and 0.03 µg mL$^{-1}$).
• Root wt% of maize at a nanosilica concentration of 15 kg ha\textsuperscript{-1} (24.29 ± 0.4 %) is higher than micro silica (5.67 %) and control (7.73 %) which may be due to the increased accumulation of silica in leaf bundle sheath.

• The raised protein content with an increase in the concentration of nano silica particularly at 15 kg ha\textsuperscript{-1} (29.08 mg g\textsuperscript{-1}) may be attributed to the existence of metabolic balance between induction of proteins such as cell wall transporters and damping off stress-responsive enzyme.

• The extent of SiO\textsubscript{2} deposition in maize leaves is found to be maximum (19.18 %) at 15 kg ha\textsuperscript{-1} when compared to micro silica (4.4 %) and control (4.4 %). This may be due to increased particle size (micron size) which is unutilised by plant.

• GC-MS analysis of the nano SiO\textsubscript{2} treated leaves shows less total organic compounds like phenols, aldehydes, etc.

• HRSEM and optical microscopic observations of maize leaf blades and roots show an increase in SiO\textsubscript{2} accumulation with the treatment of nano silica. Decrease in SiO\textsubscript{2} at 20 kg ha\textsuperscript{-1} may be due to the transition of abundantly absorbed silica to the older leaf blades. Hence, nano silica promotes the induced thickness of epidermal Si–cellulose layer which in turn supports the mechanical stability of plants.

• Eventhough, the foliar applied maize leaves show a decrease in stress with an increase in silica content, the stress tolerance achieved is not as high as the mode of soil amendment of silica.
• By investigating the responses of maize growth and physiological components to the regimes of nano and micro silica, 15 kg ha\(^{-1}\) of nano silica contributes an augmented silica accumulation and growth characteristics. Hence, there is a possibility that the nano silica at 15 kg ha\(^{-1}\) may render the optimal regime for improved growth during field application.

4.4 EFFECT OF SILICA NANOPARTICLES ON CONFERRING DISEASE RESISTANCE IN MAIZE

• Maize plantlets pre-amended with nano and micro silica sources are inoculated with fungal pathogens such as \textit{F. oxysporum} and \textit{A. niger}. The plants infected with fungi are screened for the resistance in terms of the evaluation of disease index and the expression of plant defense compounds.

• The disease incidence found in control plant is 2.67 lesions cm\(^{-1}\) while it is 2.27 lesions cm\(^{-1}\) in micro silica inoculated with \textit{F. oxysporum} but less in leaves treated with \textit{A. niger}.

• An irregular expression of the enzymes in nanosilica treated plants differs with the type of fungal infections. The PAL activity is found to be low at N15 (97.0 ± 2.41) sprayed with \textit{A. niger}. As the concentration of nano silica increases, the PAL activity tends to decrease during the infection of both fungi.

• A comparison of plants inoculated with \textit{F. oxysporum} and \textit{A. niger} shows that those inoculated with \textit{A. niger} are more
susceptible to silica treatments due to the damping-off mechanism in plants. The possible relationship between nano silica and decreased stress responsive enzyme is due to the deposition of silica that poises stress-related macromolecules.

- In this study, the degree of externally induced resistance conferred by the hydrophobic silica nanoparticles is high at 10 and 15 kg ha$^{-1}$ when compared to micro silica.

- Thus, an intrusion of nano silica via active transport is a defensible way to avoid cellular damage to pathogens in maize leaves. The observed results confirm that the properly functionalised nanoparticles may be effectively used to drop off chemicals (fungicides) on plant tissues.

### 4.5 ENHANCING THE BIOCONTROL ACTION OF MAIZE EMPLOYING SILICA NANOPARTICLES

- This investigation is to provide a better biocontrol action against fungal pathogens as well as insects affecting maize crop. The degree of physical strength attained in leaves by the treatment with nano silica is elucidated from the leaf hardness tests using nanomechanical testing systems.

- A roughness and hardness of the leaf blades treated with nano silica are respectively 1640 nm and 26.46 MPa. Nano silica is physiosorbed by the cuticular lipids, disrupting the protective barrier and causes insecticidal action purely by physical means.

- Application of biocontrol agent, P. fluorescens combined with silica sources in soil induces higher population
(4.4×10^4 CFU g^-1) of *P. fluorescens* at nano scale than control (2.6×10^4 CFU g^-1) and micro (3.2×10^4 CFU g^-1).

Therefore, it is inferred that the enhanced bacterial population by nano silica promotes the degree of biocontrol action against maize pathogen in soil.

- Moreover, a decrease in stress responsive enzyme content in the roots is noticed with an increase in the concentration of nano silica treatment which indirectly conveys lesser stress in maize.

- It is an interesting observation that total phenols and physical strength of the leaves are greatly improved to resist fungal stress. Hence, defense mechanism in monocotyledons supports the application of nano silica formulated with *P. fluorescens* to achieve a broad spectrum of biocontrol activity.

**CONCLUSIONS**

- Silica nanoparticles are synthesised from rice husk biomass and characterised using different techniques such as XRD, FTIR, SEM-EDAX, PSD, TEM and surface area analysis. The characterisation results reveal the amorphous silica nanoparticles in the particle size range of 20-40 nm with 97 % purity and a high surface area of 360.85 m^2^ g^-1^.

- In vitro study on animal cell lines (MG-63) shows non-toxic nature of silica nanoparticles up to 125 μg mL^-1^. Similarly, the total microbial populations and nutrient balance in the soil and maize rhizosphere are significantly improved by nano silica treatment.
Hydroponic culturing of maize seeds treated with different silica sources confirms the enhanced seed stability, SiO$_2$ deposition and favourable pH and conductivity under nano silica treatments.

Growth and physiological responses of maize are high at 15 kg ha$^{-1}$ of nano silica treatment than any other regimes under field conditions. Total biochemical compounds assayed from maize leaf and root extracts reveals better sustainability at the same regime. In addition, higher accumulation of silica bodies at root and leaf epidermis is evident by nano scale silica particles.

Disease index and number of lesions are found to be reduced in maize amended with silica nanoparticles which is also substantiated by the expression of stress responsive plant biochemical compounds against fungal pathogens.

Similarly, development of biocontrol mechanism in maize by silica nanoparticles is authenticated by mechanical characterisation of leaves. The resultant biological nanocomposites (P. fluorescens-nano silica) enrich biocontrol action in maize which is supported from the biochemical estimations of defense compounds.

The production of cost effective high surface silica nanoparticles will be a potential alternative to micro silica fertilizers. Moreover, a detailed molecular evaluation of silica transport mechanisms and multiple grow out test of plants treated with silica nanoparticles may find a solution to authenticate environmental applications.
• Thus, the present study provides the platform to build cost effective silica fertilizers using nanobiotechnology to improve the growth and yield of maize crop in future.

FUTURE SCOPE OF THE WORK

• Production of the efficient silica source which is economical and easily up taken by the plant is essential in the current scenario to avoid silica deficiency in soil.

• Assessment of the toxicity, plant physiological responses and disease resistance influenced by nanoscale silica will help to ascertain the detailed interaction mechanism of silica with soil and plant and hence, to facilitate the application of silica nanoparticles for real time agronomic practices.

• Sustainable crop cultivation aided by silica nanoparticles will progress the yield of food crops which requires silica sources. Development of novel agroformulation to meet the current demands of silica sources for crop improvement especially for monocotyledons like rice, maize etc.

• Hence, the present investigation will transmit the unique technology for further commercialisation of nanoscale fertilisers and different nanoparticles based agro nutrient formulations.