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Development and Substantiation of Nano Formulation with Anti-viral Phtyomolecules Derived from Wild Papaya Genotypes for Extenuation of Papaya Ring Spot Virus (PRSV)

S. Ramya, J. Auxcilia[1]*, Jeya Sundara Sharmila D[2] and K. Hemaprabha K[3]

Department of Fruit Science, HC&RI, [1]Directorate of Extension Education,
[2]Centre for Nano science and Technology, TNAU, Coimbatore-641 003, India
[3]Forest College and Research Institute, TNAU, Mettupalayam-641 301, India
E-mail: auxcilia@tnau.ac.in

***Abstract*:** Octadecanoic acid is a bioactive compound, a component of triglycerides reported to be present in *V. candamarcensis* and *V. cauliflora* which might have anti-viral activity against PRSV in papaya. The present study focused on the synthesis of nano emulsion of bioactive compounds using methods such as High energy homogenization and Sonication. The nano emulsion showed z-average diameter of 52.6 nm as spherical shaped droplet structure which was confirmed in TEM imaging. Plant bioassay was carried out for assessing the resistance activity of nano emulsion against papaya ring spot virus in papaya under a glasshouse condition using mechanical inoculation methods, in which pre-inoculation and co-inoculation methods with nano emulsion did not exhibit any symptoms of papaya ring spot virus infection in papaya seedlings. But in the post-inoculation method, treatments with Octadecanoic acid nano formulation had symptoms of papaya ring spot virus infection in papaya seedlings. Further studies are required to confirm the effective concentration of nano formulation of bioactive compounds for the control of PRSV infection at different growth stages.

***Keywords*:** Bioactive compounds, Octadecanoic acid, Nano-emulsion, Mechanical inoculation

Papaya (*Carica papaya* L.), a member of the Caricaceae family, has been planted in home gardens for centuries. Later on, it became a commercial and industrial crop due to the widespread usage of papain in the pharmaceutical and cosmetics sectors (Auxcilia et al 2020). Presently worldwide papaya production is threatened by the most destructive disease called papaya ring spot disease (Premchand et al 2021). Around the world, numerous initiatives, including a transgenic strategy, have been attempted to curb the PRSV incidence in papaya. Therefore, management of PRSV is required to reduce disease incidence and yield loss. Gohilapriya et al (2021) revealed the presence of a few bioactive compounds in wild genotypes of papaya such as *Vasconcellea cauliflora* and *Vasconcellea candamarcensis*, while it was absent in cultivated varieties. Among which, the compounds Octadecanoic acid and other phytomolecules were found in wild genotypes *viz., Vasconcelleae candamarcensis* and *Vasconcelleae cauliflora* had significance in possessing anti-viral properties. These compounds are reported to possess anti-viral activity against plant viruses. Gas chromatography–mass spectrometry (GC/MS) analysis was used to identify the existence of volatile antifungal chemicals in the bacterial secretome (Nakkeer*an* et al 2020). The antifungal chemicals pentadecanoic acid, n-hexadecanoic acid, octadecanoic acid (stearic acid) and tetradecanoic acid exhibited an increase in peak area with response to antifungal potential therapy. However, the above compounds are not hydrophilic in nature so that there is a need to convert them into hydrophilic for the applications in plants. Nanotechnology has significantly improved plant pathogen detection, crop protection, disease management, nutrient loss reduction in fertilization, plant germination, growth and plant-pathogen control in agricultural research. The development of nano chemicals has the potential to improve fertilizers, insecticides and plant growth. Plants use a variety of delivery systems, which include nanoparticles, nano capsules and nanoemulsions (Dutta et al 2022).

MATERIAL AND METHODS

High pressure homogenization: The microemulsion prepared was fed into high pressure homogenizer at high pressure for 5 to 10 cycles for homogenization.

Ultrasonication: Using a 24 KHz sonicator (Dr. Hielscher series, Model UP 400S), emulsification by sonication was done. The duration fixed was 30 minutes (ON/OFF for 5 seconds) with an amplitude of 60% and a temperature of 25°C.

Characterization of nanoparticles: The nanoemulsions were characterized using Particle Size Analyzer (PSA), Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM).

Experimental details: Bioassay study was conducted at glasshouse, Department of Fruit Science, Tamil Nadu Agricultural University, Coimbatore. The assay was carried out by using completely randomized design (CRD) with eight treatments and with concentrations from 10-100 ppm.

Statistical analysis: The data collected during this research were pooled and analyzed using the MS Excel program and were statistically analyzed using R- Studio, AGRES software (0.74.0.0 version).

RESULTS AND DISCUSSION

The nano formulation of octadecanoic acid characterized in PSA for particle size has a mean droplet size of 313.9 nm, the zeta potential of 65.4 mV, and poly dispersity index of 0.398. This study technique produced more size reduction, poly dispersity index and zeta potential than previous research by Kumar and Randhawa (2015) that demonstrated the size of stearic acid solid lipid nanoparticles (SASLNs) greatly depends on the concentration of Gelucire® 50/13 in the solid lipid nano formulation with zeta average particle size of 1700 nm. The stretching of the methylene group (CH_3) in octadecanoic acid nano emulsion was represented by the peak 2937cm^{-1} which was similar to the FT-IR results of Neg*i* et al (2014). The findings of TEM showed that the Octadecanoic acid nano formulation was having a spherical shape droplets with the nano range of (52.6 nm). Earlier Cryo-TEM method was incorporated to validate the particle size as well as to characterize the stearic acid nano emulsion shape and structure, reported that their micrographs obtained using an Ultras can XP camera at a nominal magnification of 25,000x and a defocus of 200nm showing well-defined spherical shapes of the droplets in the nano-system of astaxanthin encapsulated in stearic acid nano emulsion (Flores-Miran*da* et al 2020). The SEM image of octadecanoic acid nano formulation confirmed that they are spherical. From the SEM images of the octadecanoic acid, it could be concluded that the formulation was in the nano range of 644.1 nm which was similar to the report of (W*ang* et al 2011) where SEM was used to examine the surface morphology of copper foils with stearic acid and numerous nanosheets with various shapes are randomly dispersed across the surface with the flexible micro-nano sheet thickness of around 500 nm.

The symptoms were measured at two intervals on the 15th and 30th days after pre-inoculation, post-inoculation and co-inoculation (Sangeetha et al 2020). On15th and 30th day of pre-inoculation and co-inoculation, there were no symptoms observed on the plants, The absence of symptoms in the present study might be due to the inhibitory effects on DNA replication and antigen production by octadecanoic acid nano formulation. Li et al (2010) elaborated the delivery strategy of stearic acid-g-chitosan oligosaccharide polymeric micelles for lamivudine stearate and its antiviral activity by, in which the prodrug of lamivudine (Ia), lamivudine stearate , was produced via ester linkage between LA and stearic acid to boost the lipophilicity of a water-soluble antiviral drug. When compared to LA and LAS, Stearic acid-g-chitosan oligosaccharide (CSO-SA) on *in vitro* showed anti-HBV (Hepatitis B Virus) actions with pronounced inhibitory effects on DNA replication and antigen production.

The symptoms on 15th day of post-inoculation showed that the percentage of disease severity was the highest in treatment T_8 (Inoculated control) with 30.5 % followed by the treatment T_1 (stearic acid 10 ppm) 29.4 % and lowest in treatment T_5(stearic acid 100 ppm) with 20.3 % on 30th day of post-inoculation. The disease severity was highest in treatment T_8 (inoculated control) with 56.3 % followed by treatment T_1 with 47.3 % and lower in treatment T_5 with 39.2

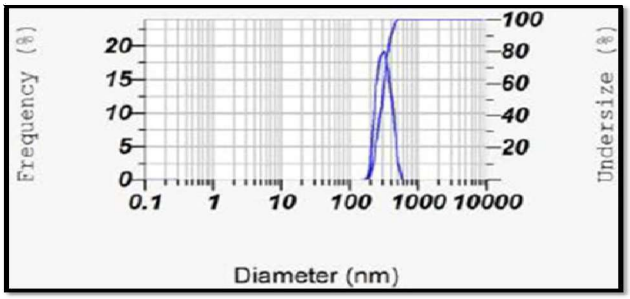

Fig. 1. Size of octadecanoic acid nano formulation during synthesis

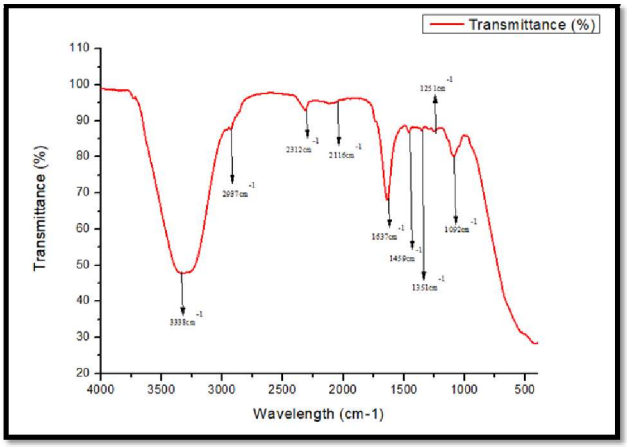

Fig. 2. FTIR spectrum of Octadecanoic acid nano emulsion

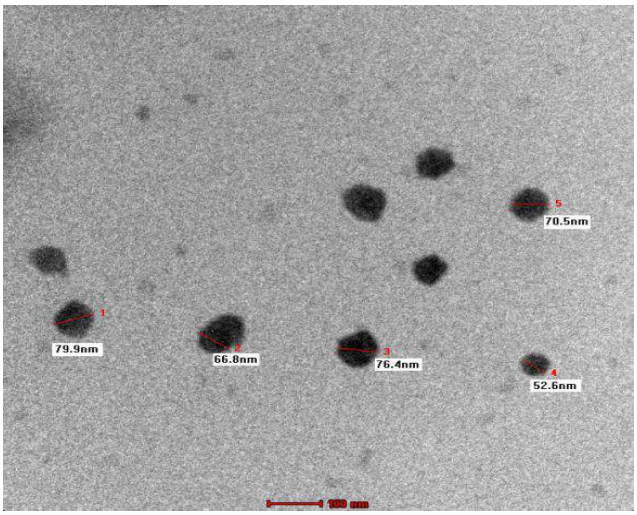

Fig. 3. TEM image of Octadecanoic acid nano emulsion

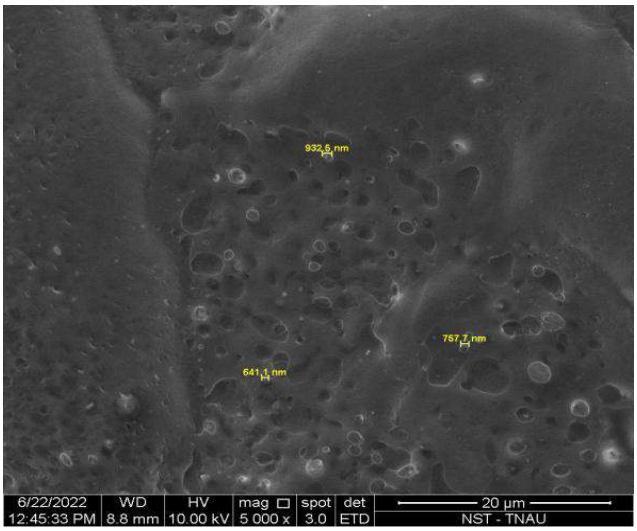

Fig. 4. SEM image of Octadecanoic acid nano emulsion

Table 1. Assessment of PRSV disease severity with application of octadecanoic acid nano formulation

Treatment (Days)	Post-inoculation (%)	
	15th (DAI)	30th (DAI)
T1	29.4	47.3
T2	27.8	45.7
T3	25.9	41.2
T4	25.2	39.4
T5	20.3	39.2
T6	0	0
T7	0	0
T8	30.5	56.3
Mean	19.8	32.3
CD (p= 0.05)	1.12	1.45

%. The concentration of 100ppm of octadecanoic acid nano formulation spray had maximum effect on plant height, leaf area, stem girth and the number of leaves in all inoculation methods Xiong et al (2020) observed that octadecanoic acid possesses not only antiviral properties but also antibacterial, antimicrobial and antifungal properties in plants.

CONCLUSION

The study proved the efficacy of nano formulations of bioactive compounds derived from wild papaya genotypes in inducing resistance against PRSV infection in domesticated papaya plants (TNAU papaya CO.8) that are vulnerable to PRSV. Moreover, the biosafety of the nano formulation was ensured through dose specific threshold limit of maximum 100 ppm while the efficacy was registered from 10 ppm onwards and the study must be continued for molecular level analysis and field trials to confirm the efficacy of bioactive compounds nano formulation against the papaya ring spot virus.

AUTHORSHIP CONTRIBUTION

Ramya S: Investigation, Writing-original draft, J. Auxcilia: conceptualization, methodology, validation, investigation, Jeya Sundara Sharmila: methodology for nanotechnology, K. Hemaprabha: resources, K.S. Subramanian: technical support for nanotechnology.

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